Figure 1

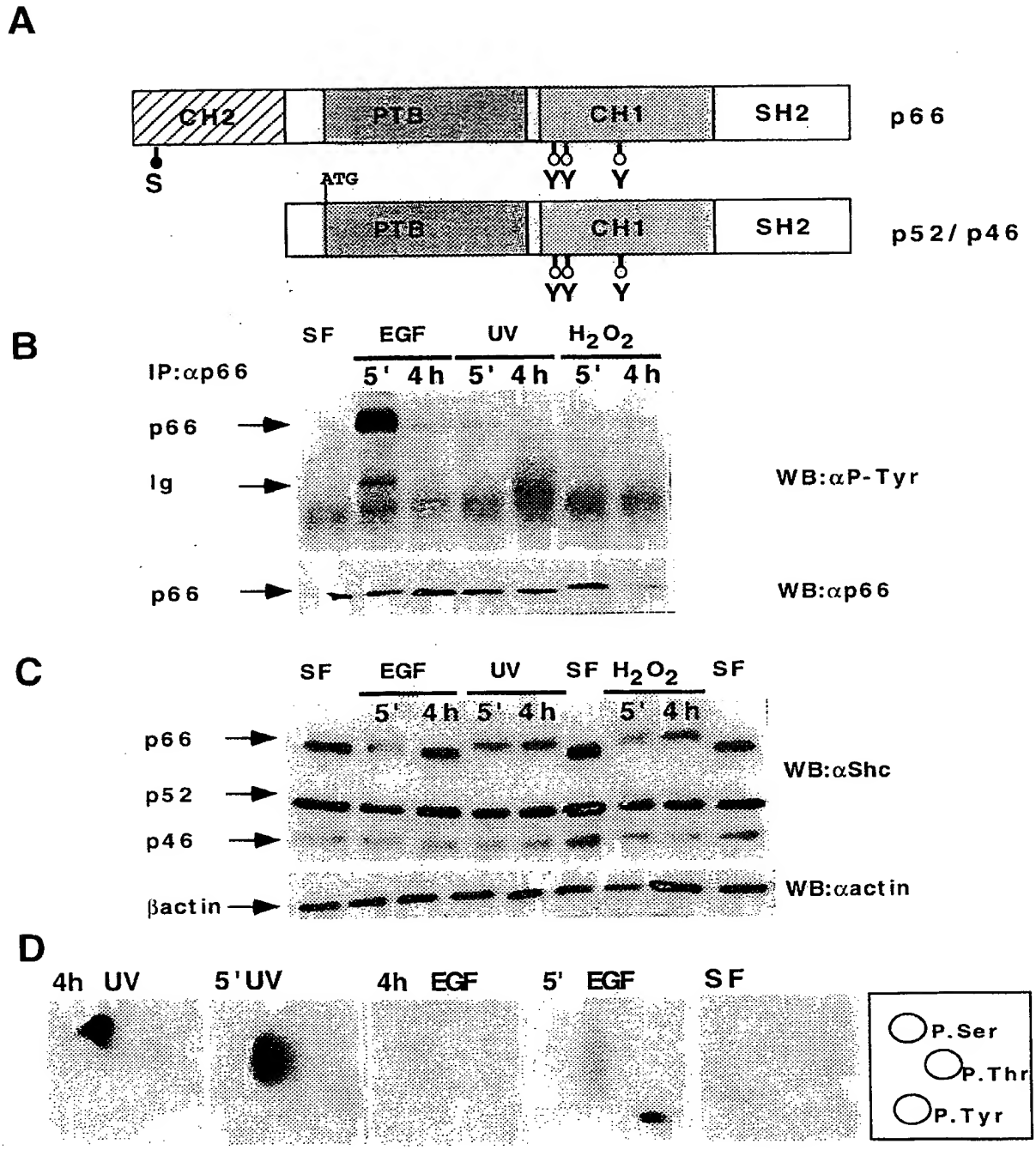
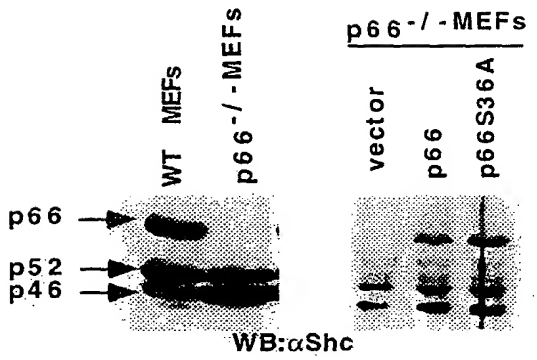
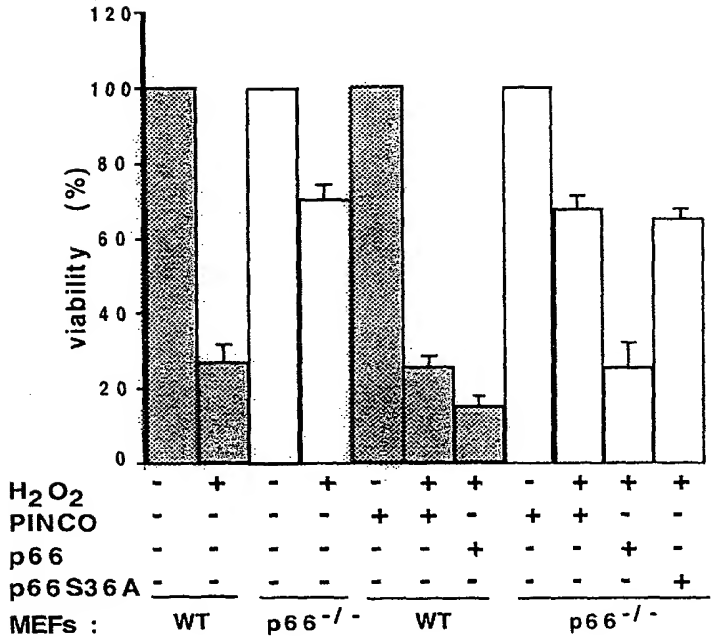

A
CH2
PTB
CH1
SH2
p66
S
ATG
YY
Y
PTB
CH1
SH2
p52/ p46
YY
Y
B
SF
EGF
UV
H_2O_2
IP:αp66
5' 4h 5' 4h 5' 4h
p66
Ig
WB:αP-Tyr
p66
WB:αp66
C
SF
EGF
UV
SF
H_2O_2
SF
5' 4h 5' 4h 5' 4h
p66
p52
p46
WB:αShc
βactin
WB:αactin
D
4h UV
5'UV
4h EGF
5' EGF
SF
P.Ser
P.Thr
P.Tyr

Figure 2

A


WT MEFs
p66-/- MEFs
p66-/- MEFs
vector
p66
p66S36A
p66
p52
p46
WB:αShc

B


viability (%)
120
100
80
60
40
20
0
H2O2
PINCO
p66
p66S36A
MEFs :
WT
p66-/-
WT
p66-/-

C

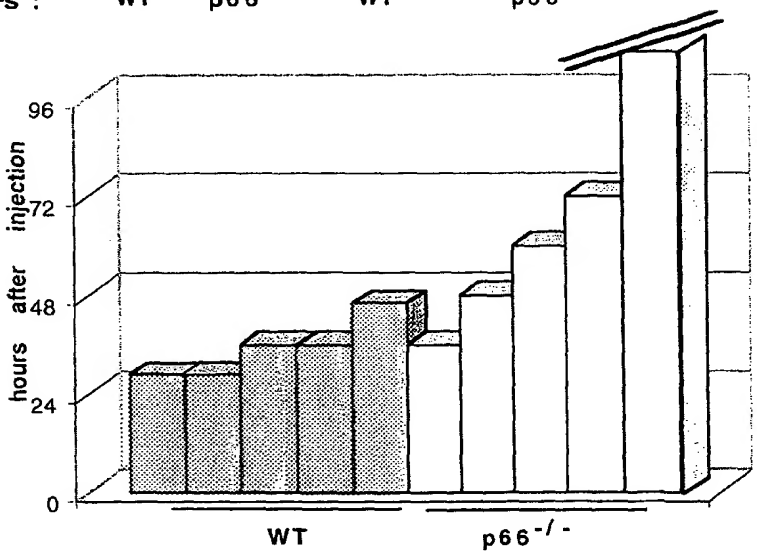

hours after injection
96
72
48
24
0
WT
p66-/-

Figure 3

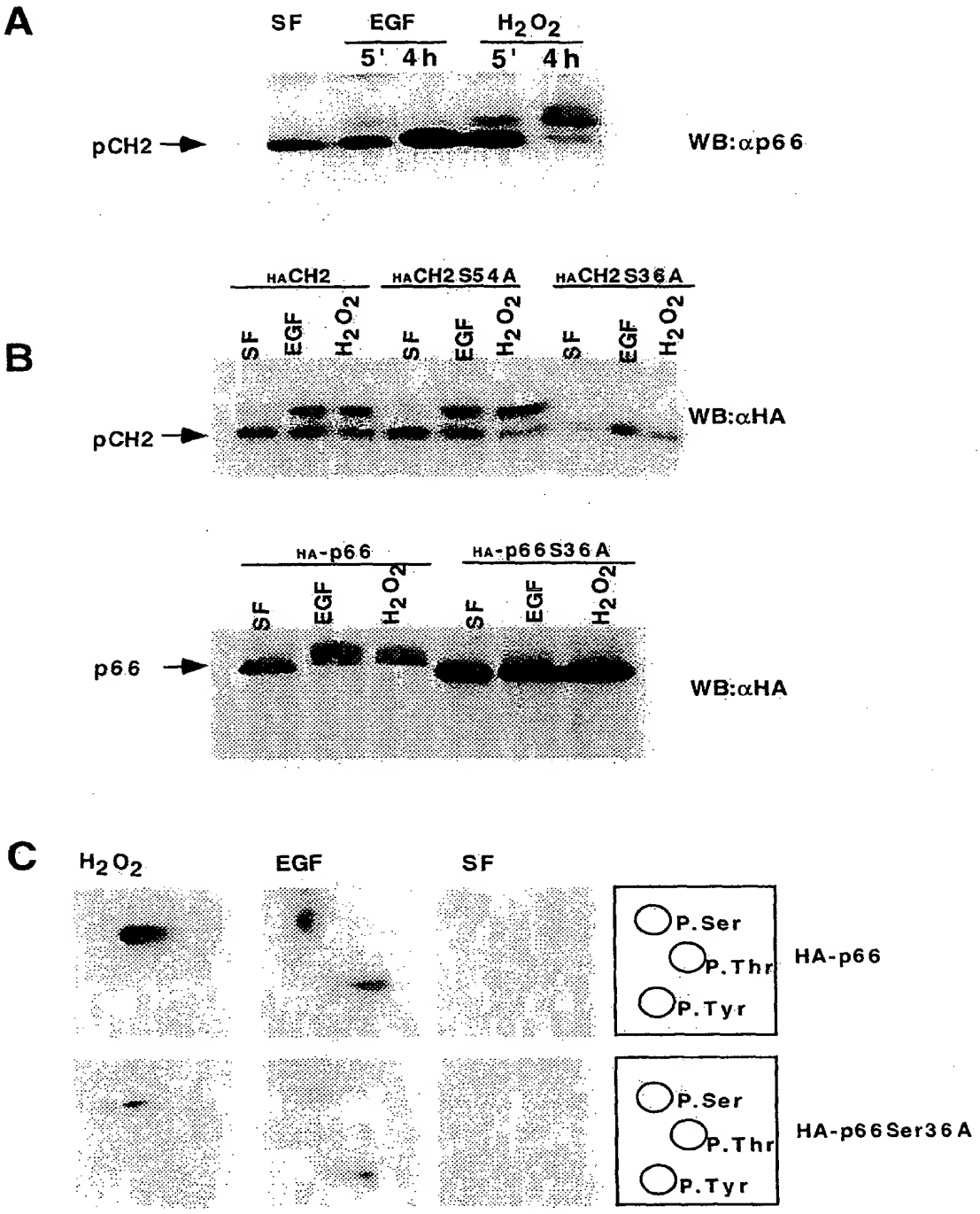

A
SF
EGF
H_2O_2
5' 4h 5' 4h
pCH2
WB:αp66
B
HACH2
HACH2S54A
HACH2S36A
SF EGF H_2O_2 SF EGF H_2O_2 SF EGF H_2O_2
pCH2
WB:αHA
HA-p66
HA-p66S36A
SF EGF H_2O_2 SF EGF H_2O_2
p66
WB:αHA
C
H_2O_2
EGF
SF
P.Ser
P.Thr
P.Tyr
HA-p66
HA-p66Ser36A

Figure 4

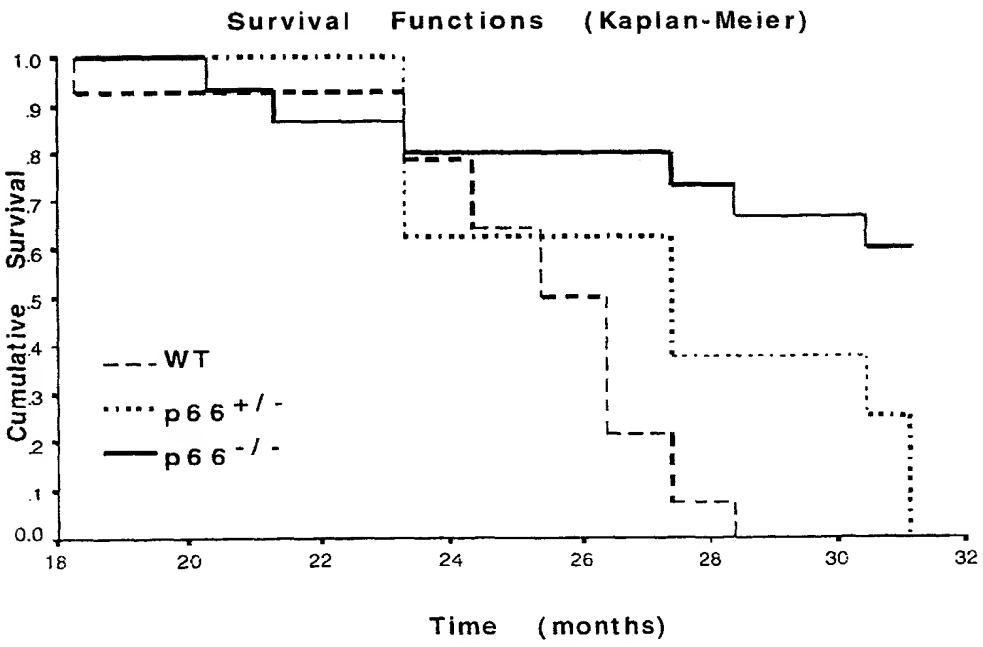

Survival Functions (Kaplan-Meier)
Cumulative Survival
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0.0
18
20
22
24
26
28
30
32
WT
p66+/-
p66-/-
Time (months)

Figure 5a

```
        10         20         30         40         50         60
atggggcctg aaactgtctg ggtctgagct ggggagcgga agccacttgt ccctctccct

        70         80         90        100        110        120
ccccaggact tctgtgactc ctgggccaca gaggtccaac cagggtaagg gcctggggat

       130        140        150        160        170        180
acccccctgcc tggccccctt gcccaaactg gcaggggggc caggctgggc agcagccccc

       190        200        210        220        230        240
ctttcacctc aactATGGAT CTCCTGCCCC CCAAGCCCAA GTACAATCCA CTCCGGAATG

       250        260        270        280        290        300
AGTCTCTGTC ATCGCTGGAG GAAGGGGCTT CTGGGTCCAC CCCCCCGGAG GAGCTGCCTT

       310        320        330        340        350        360
CCCCATCAGC TTCATCCCTG GGGCCCATCC TGCCTCCTCT GCCTGGGGAC GATAGTCCCA

       370        380        390        400        410        420
CTACCCTGTG CTCCTTCTTC CCCCGGATGA GCAACCTGAG GCTGGCCAAC CCGGCTGGGG

       430        440        450        460        470        480
GGCGCCCAGG GTCTAAGGGG GAGCCAGGAA GGGCAGCTGA TGATGGGGAG GGGATCGATG

       490        500        510        520        530        540
GGGCAGCCAT GCCAGAGTCA GGCCCCCTAC CCCTCCTCCA GGACATGAAC AAGCTGAGTG

       550        560        570        580        590        600
GAGGCGGCGG GCGCAGGACT CGGGTGGAAG GGGGCCAGCT TGGGGGCGAG GAGTGGACCC

       610        620        630        640        650        660
GCCACGGGAG CTTTGTCAAT AAGCCCACGC GGGGCTGGCT GCATCCCAAC GACAAAGTCA

       670        680        690        700        710        720
TGGGACCCGG GGTTTCCTAC TTGGTTCGGT ACATGGGTTG TGTGGAGGTC CTCCAGTCAA

       730        740        750        760        770        780
TGCGTGCCCT GGACTTCAAC ACCCGGACTC AGGTCACCAG GGAGGCCATC AGTCTGGTGT

       790        800        810        820        830        840
GTGAGGCTGT GCCGGGTGCT AAGGGGGCGA CAAGGAGGAG AAAGCCCTGT AGCCGCCCGC

       850        860        870        880        890        900
TCAGCTCTAT CCTGGGGAGG AGTAACCTGA AATTTGCTGG AATGCCAATC ACTCTCACCG

       910        920        930        940        950        960
TCTCCACCAG CAGCCTCAAC CTCATGGCCG CAGACTGCAA ACAGATCATC GCCAACCACC

       970        980        990       1000       1010       1020
ACATGCAATC TATCTCATTT GCATCCGGCG GGGATCCGGA CACAGCCGAG TATGTCGCCT

      1030       1040       1050       1060       1070       1080
ATGTTGCCAA AGACCCTGTG AATCAGAGAG CCTGCCACAT TCTGGAGTGT CCCGAAGGGC

      1090       1100       1110       1120       1130       1140
TTGCCCAGGA TGTCATCAGC ACCATTGGCC AGGCCTTCGA GTTGCGCTTC AAACAATACC

      1150       1160       1170       1180       1190       1200
TCAGGAACCC ACCCAAACTG GTCACCCCTC ATGACAGGAT GGCTGGCTTT GATGGCTCAG

      1210       1220       1230       1240       1250       1260
CATGGGATGA GGAGGAGGAA GAGCCACCTG ACCATCAGTA CTATAATGAC TTCCCGGGGA

      1270       1280       1290       1300       1310       1320
AGGAACCCCC CTTGGGGGGG GTGGTAGACA TGAGGCTTCG GGAAGGAGCC GCTCCAGGGG

      1330       1340       1350       1360       1370       1380
CTGCTCGACC CACTGCACCC AATGCCCAGA CCCCCAGCCA CTTGGGAGCT ACATTGCCTG
```

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Figure 5a continued

```
      1390        1400        1410        1420        1430        1440
TAGGACAGCC TGTTGGGGGA GATCCAGAAG TCCGCAAACA GATGCCACCT CCACCACCCT

      1450        1460        1470        1480        1490        1500
GTCCAGGCAG AGAGCTTTTT GATGATCCCT CCTATGTCAA CGTCCAGAAC CTAGACAAGG

      1510        1520        1530        1540        1550        1560
CCCGGCAAGC AGTGGGTGGT GCTGGGCCCC CCAATCCTGC TATCAATGGC AGTGCACCCC

      1570        1580        1590        1600        1610        1620
GGGACCTGTT TGACATGAAG CCCTTCGAAG ATGCTCTTCG GGTGCCTCCA CCTCCCCAGT

      1630        1640        1650        1660        1670        1680
CGGTGTCCAT GGCTGAGCAG CTCCGAGGGG AGCCCTGGTT CCATGGGAAG CTGAGCCGGC

      1690        1700        1710        1720        1730        1740
GGGAGGCTGA GGCACTGCTG CAGCTCAATG GGGACTTCTT GGTACGGGAG AGCACGACCA

      1750        1760        1770        1780        1790        1800
CACCTGGCCA GTATGTGCTC ACTGGCTTGC AGAGTGGGCA GCCTAAGCAT TTGCTACTGG

      1810        1820        1830        1840        1850        1860
TGGACCCTGA GGGTGTGGTT CGGACTAAGG ATCACCGCTT TGAAAGTGTC AGTCACCTTA

      1870        1880        1890        1900        1910        1920
TCAGCTACCA CATGGACAAT CACTTGCCCA TCATCTCTGC GGGCAGCGAA CTGTGTCTAC

      1930        1940        1950        1960        1970        1980
AGCAACCTGT GGAGCGGAAA CTGtgatctg ccctagcgct ctcttccaga agatgccctc

      1990        2000        2010        2020        2030        2040
caatcctttc caccctattc cctaactctc gggacctcgt ttgggagtgt tctgtgggct

      2050        2060        2070        2080        2090        2100
tggccttgtg tcagagctgg gagtagcatg gactctgggt ttcatatcca gctgagtgag

      2110        2120        2130        2140        2150        2160
agggtttgag tcaaaagcct gggtgagaat cctgcctctc cccaaacatt aatcaccaaa

      2170        2180        2190        2200        2210        2220
gtattaatgt acagagtggc ccctcacctg ggcctttcct gtgccaacct gatgcccctt

      2230        2240        2250        2260        2270        2280
ccccaagaag gtgagtgctt gtcatggaaa atgtcctgtg gtgacaggcc cagtggaaca

      2290        2300        2310        2320        2330        2340
gtcacccttc tgggcaaggg ggaacaaatc acacctctgg gcttcagggt atcccagacc

      2350        2360        2370        2380        2390        2400
cctctcaaca cccgcccccc ccatgtttaa actttgtgcc tttgaccatc tcttaggtct

      2410        2420        2430        2440        2450        2460
aatgatattt tatgcaaaca gttcttggac ccctgaattc ttcaatgaca gggatgccaa

      2470        2480        2490        2500        2510        2520
caccttcttg gcttctggga cctgtgttct tgctgagcac cctctccggt ttgggttggg

      2530        2540        2550        2560        2570        2580
ataacagagg caggagtggc agctgtcccc tctccctggg gatatgcaac ccttagagat

      2590        2600        2610        2620        2630        2640
tgccccagag ccccactccc ggccaggcgg gagatggacc cctcccttgc tcagtgcctc

      2650        2660        2670        2680        2690        2700
ctggccgggg cccctcaccc caaggggtct gtatatacat ttcataaggc ctgccctccc

      2710        2720        2730        2740        2750        2760
```

Figure 5a continued

atgttgcatg cctatgtact ctgcgccaaa gtgcagccct tcctcctgaa gcctctgccc

2770	2780	2790	2800	2810	2820
tgcctccctt	tctgggaggg	cggggtgggg	gtgactgaat	ttgggcctct	tgtacagtta
2830	2840	2850	2860	2870	2880
actctcccag	gtggattttg	tggaggtgag	aaaaggggca	ttgagactat	aaagcagtag
2890	2900	2910	2920	2930	2940
acaatcccca	cataccatct	gtagagttgg	aactgcattc	ttttaaagtt	ttatatgcat
2950	2960	2970	2980	2990	3000
atattttagg	gctgctagac	ttactttcct	attttctttt	ccattgctta	ttcttgagca
3010	3020	3030	3040	3050	3060
caaaatgata	atcaattatt	acatttatac	atcacctttt	tgacttttcc	aagccctttt
3070	3080	3090	3100	3110	3120
acagctcttg	gcattttcct	cgcctaggcc	tgtgaggtaa	ctgggatcgc	acctttttata
3130	3140	3150	3160	3170	3180
ccagagacct	gaggcagatg	aaatttattt	ccatctagga	ctagaaaaac	ttgggtctct
3190	3200	3210	3220	3230	3240
taccgcgaga	ctgagaggca	gaagtcagcc	cgaatgcctg	tcagtttcat	ggaggggaaa
3250	3260	3270	3280	3290	3300
cgcaaaacct	gcagttcctg	agtaccttct	acaggcccgg	cccagcctag	gcccggggtg
3310	3320	3330	3340	3350	3360
gccacaccac	agcaagccgg	ccccccctct	tttggccttg	tggataaggg	agagttgacc
3370	3380	3390	3400	3410	3420
gttttcatcc	tggcctcctt	ttgctgtttg	gatgtttcca	cgggtctcac	ttataccaaa
3430	3440	3450	3460	3470	3480
gggaaaactc	ttcattaaag	tccgtatttc	ttctaaaaaa	aaaaaaaaaa	aaatacattt
3490	3500	3510	3520	3530	3540
atacatcacc	tttttgactt	ttccaagccc	ttttacagct	cttggcattt	tcctcgccta
3550	3560	3570	3580	3590	3600
ggcctgtgag	gtaactggga	tcgcaccttt	tataccagag	acctgaggca	gatgaaattt
3610	3620	3630	3640	3650	3660
atttccatct	aggactagaa	aaacttgggt	ctcttaccgc	gagactgaga	ggcagaagtc
3670	3680	3690	3700	3710	3720
agcc.....	.....	.....	.....	.....	.....

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Figure 5b

```
        10         20         30         40         50         60
MDLLPPKPKY NPLRNESLSS LEEGASGSTP PEELPSPSAS SLGPILPPLP GDDSPTTLCS

        70         80         90        100        110        120
FFPRMSNLRL ANPAGGRPGS KGEPGRAADD GEGIDGAAMP ESGPLPLLQD MNKLSGGGGR

       130        140        150        160        170        180
RTRVEGGQLG GEEWTRHGSF VNKPTRGWLH PNDKVMGPGV SYLVRYMGCV EVLQSMRALD

       190        200        210        220        230        240
FNTRTQVTRE AISLVCEAVP GAKGATRRRK PCSRPLSSIL GRSNLKFAGM PITLTVSTSS

       250        260        270        280        290        300
LNLMAADCKQ IIANHHMQSI SFASGGDPDT AEYVAYVAKD PVNQRACHIL ECPEGLAQDV

       310        320        330        340        350        360
ISTIGQAFEL RFKQYLRNPP KLVTPHDRMA GFDGSAWDEE EEEPPDHQYY NDFPGKEPPL

       370        380        390        400        410        420
GGVVDMRLRE GAAPGAARPT APNAQTPSHL GATLPVGQPV GGDPEVRKQM PPPPPCPGRE

       430        440        450        460        470        480
LFDDPSYVNV QNLDKARQAV GGAGPPNPAI NGSAPRDLFD MKPFEDALRV PPPPQSVSMA

       490        500        510        520        530        540
EQLRGEPWFH GKLSRREAEA LLQLNGDFLV RESTTTPGQY VLTGLQSGQP KHLLLVDPEG

       550        560        570        580
VVRTKDHRFE SVSHLISYHM DNHLPIISAG SELCLQQPVE RKL*
```

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Figure 6

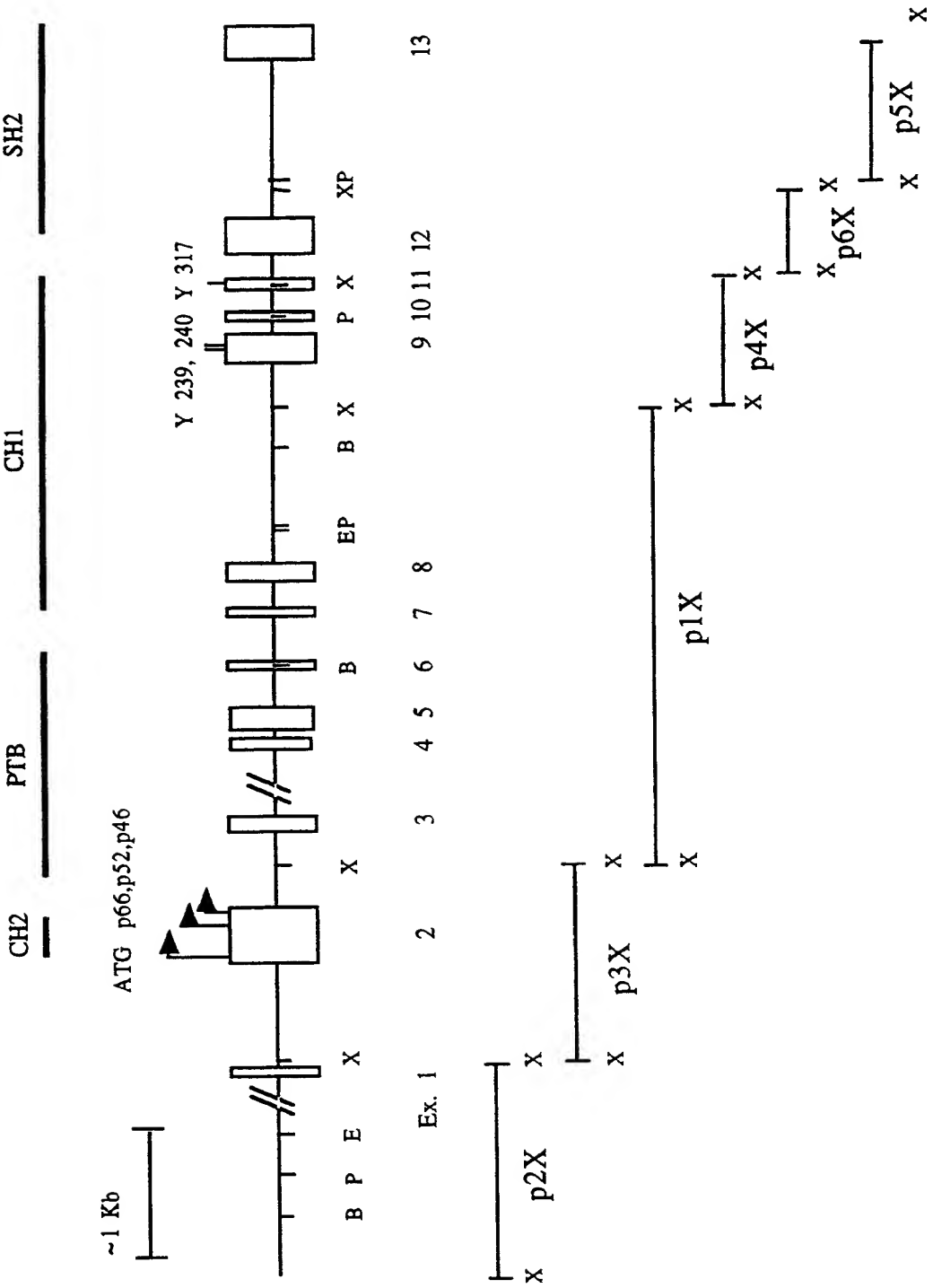

SH2
CH1
PTB
CH2
ATG p66,p52,p46
~1 Kb
B P E X
Ex. 1 2 3 4 5 6 7 8 9 10 11 12 13
X B EP B X P X XP
Y 239, 240 Y 317
p2X
p3X
p1X
p4X
p6X
p5X

Figure 7

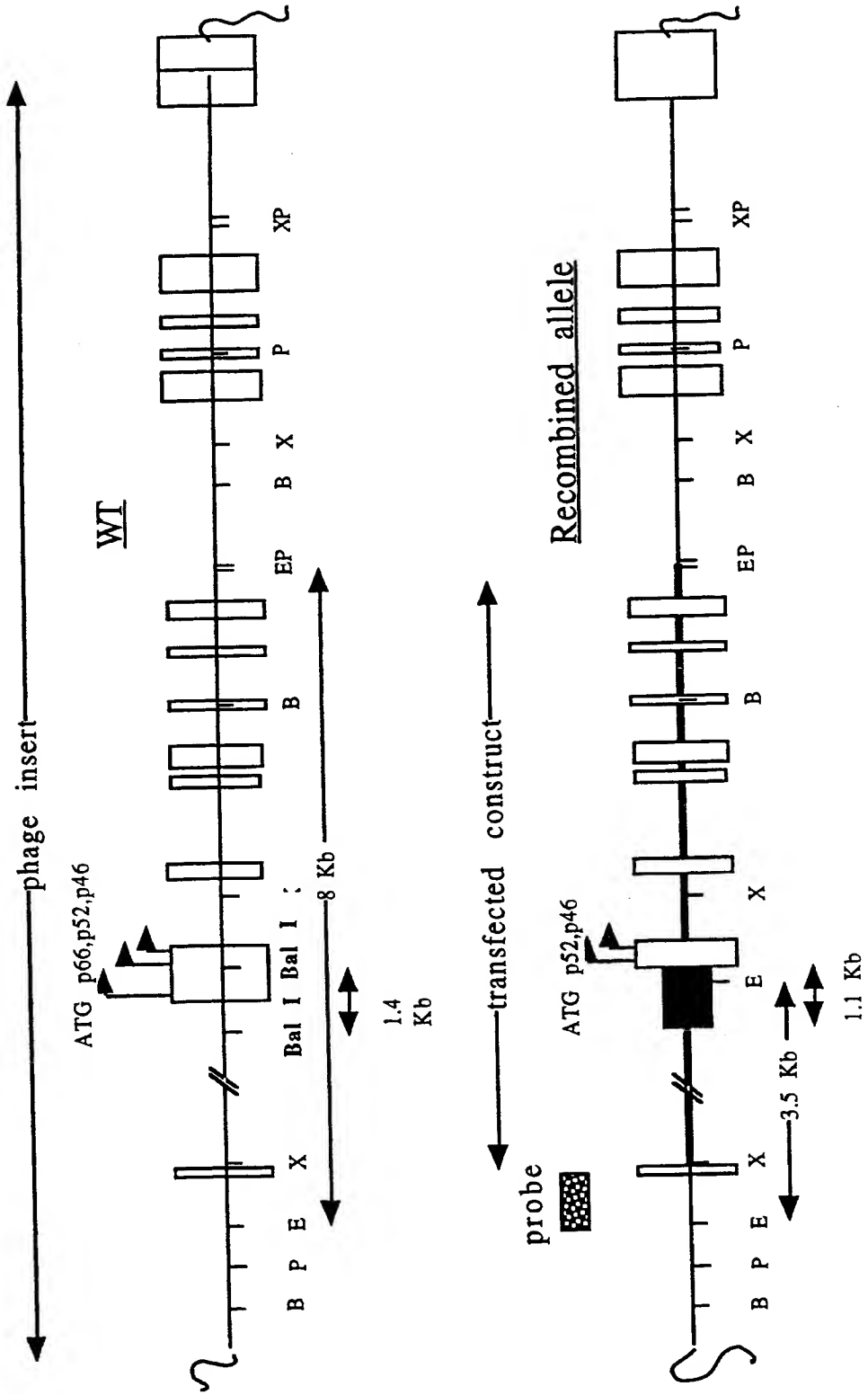
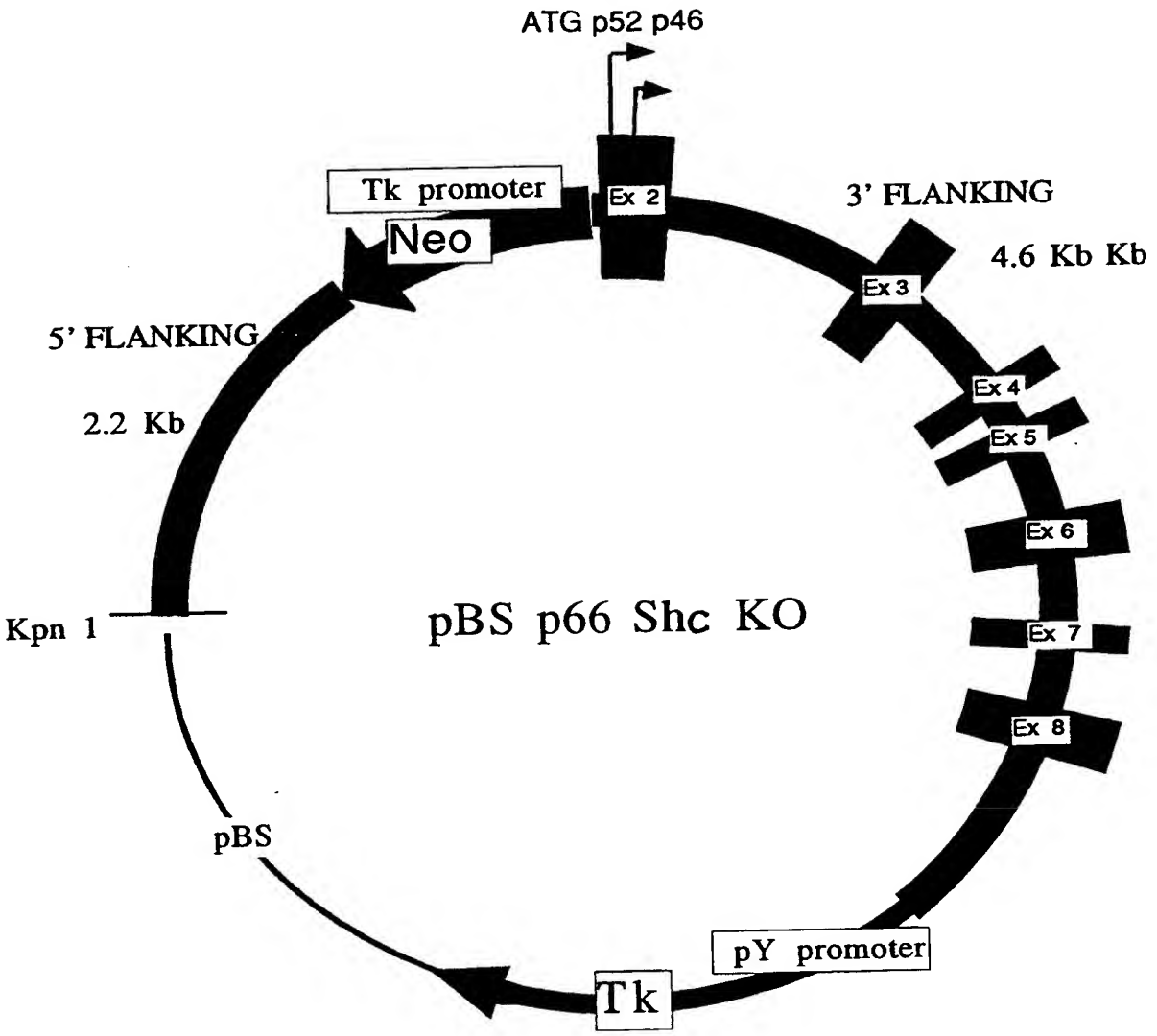

phage insert
WT
ATG p66,p52,p46
B P E X Bal I Bal I B EP B X P XP
8 Kb
1.4 Kb
transfected construct
Recombined allele
probe
ATG p52,p46
B P E X E B EP B X P XP
3.5 Kb
1.1 Kb

Figure 8


ATG p52 p46
Tk promoter
Neo
Ex 2
3' FLANKING
4.6 Kb Kb
Ex 3
Ex 4
Ex 5
Ex 6
Ex 7
Ex 8
5' FLANKING
2.2 Kb
Kpn 1
pBS p66 Shc KO
pBS
pY promoter
Tk

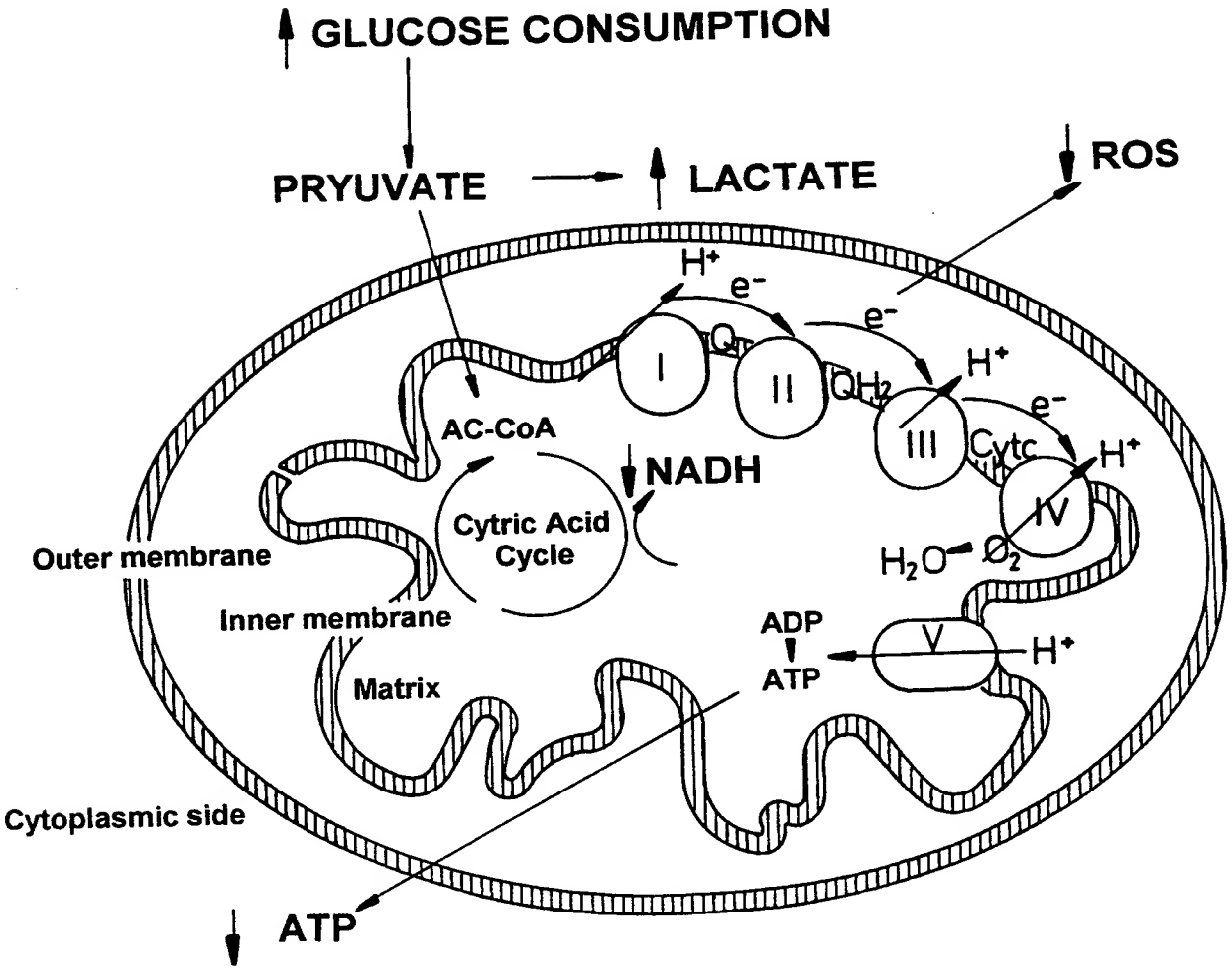

GLUCOSE CONSUMPTION
PRYUVATE
LACTATE
ROS
H+
e-
e-
I
Q
II
QH2
H+
e-
III
Cytc
H+
IV
AC-CoA
NADH
Cytric Acid Cycle
Outer membrane
Inner membrane
Matrix
H2O
O2
ADP
ATP
V
H+
Cytoplasmic side
ATP

Fig. 9

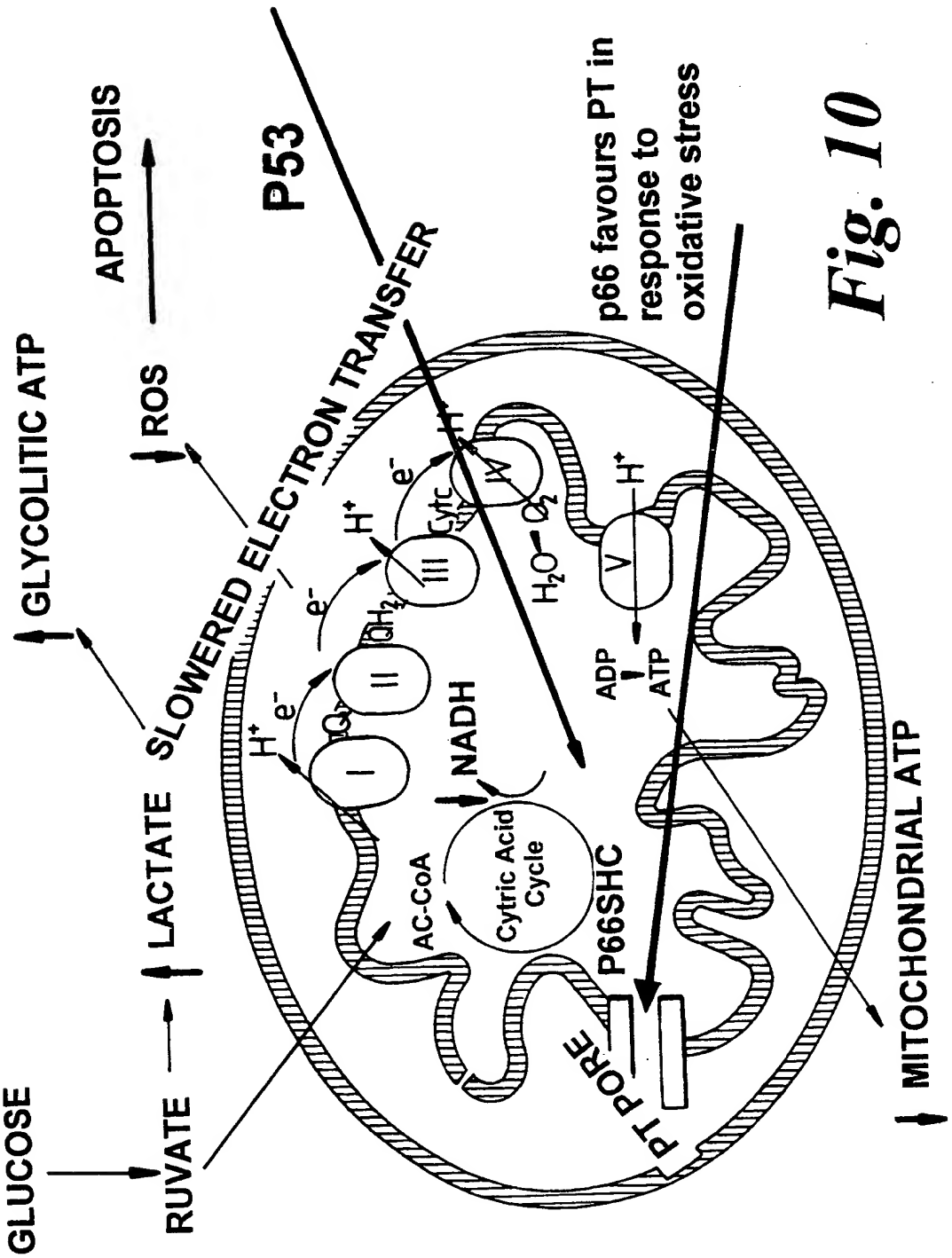

GLUCOSE
RUVATE
LACTATE
GLYCOLITIC ATP
APOPTOSIS
ROS
P53
SLOWERED ELECTRON TRANSFER
p66 favours PT in response to oxidative stress
NADH
AC-CoA
Cytric Acid Cycle
P66SHC
PT PORE
ADP
ATP
MITOCHONDRIAL ATP
H+
e-
Cytc
H2O
O2

Fig. 10

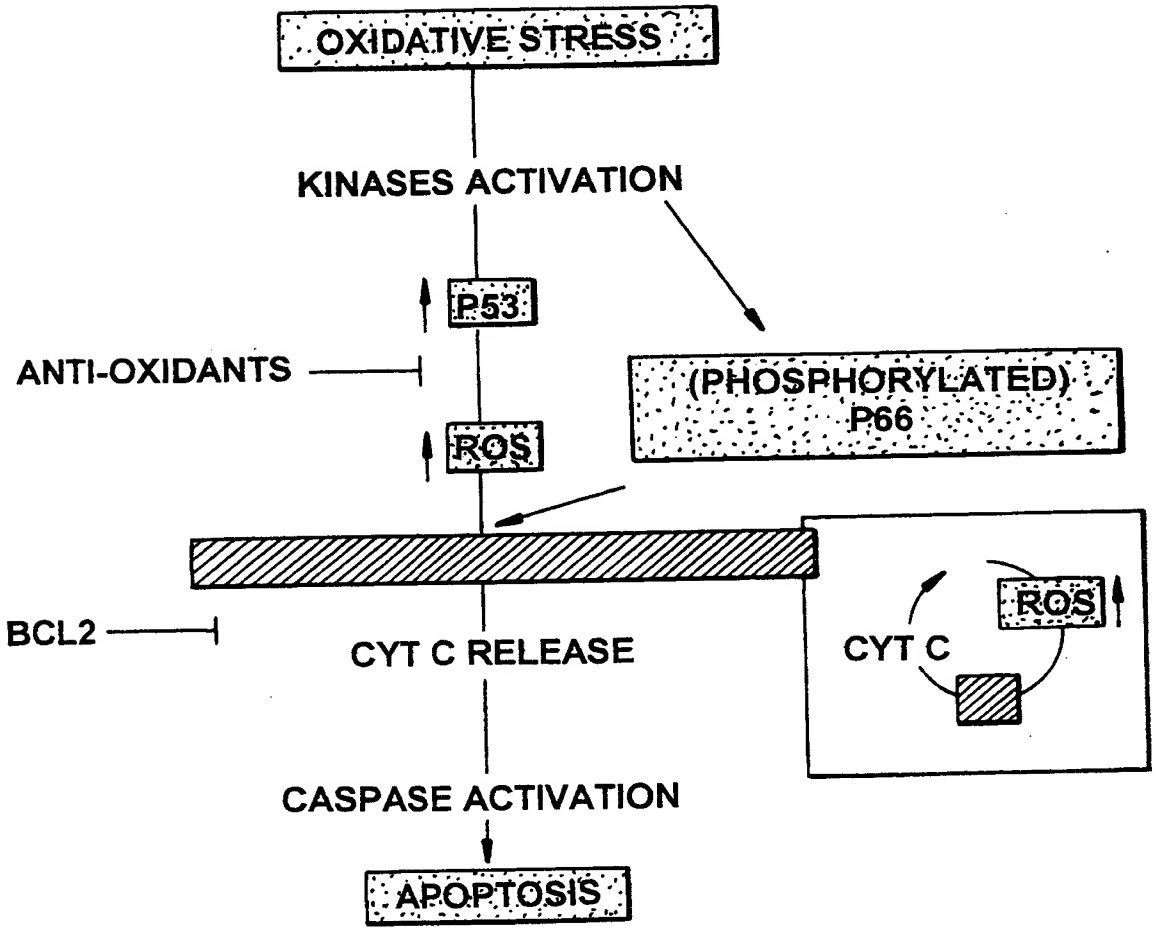

OXIDATIVE STRESS
KINASES ACTIVATION
P53
ANTI-OXIDANTS
(PHOSPHORYLATED) P66
ROS
BCL2
CYT C RELEASE
CYT C
ROS
CASPASE ACTIVATION
APOPTOSIS

Fig. 11